Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

SYNTHESIS OF SOME AROMATIC ESTERS OF THE METHYL D-GLUCOPYRANOSIDES, AND THEIR STABILITY TO HIGH-ENERGY RADIATION

ILA M. SARKAR* AND JETT C. ARTHUR, Jr.

Southern Regional Research Laboratory, Southern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture, New Orleans, Louisiana 70119 (U. S. A.)

(Received September 22nd, 1967)

ABSTRACT

The esters obtained by complete esterification of methyl α-D-glucopyranoside with each of eight differently substituted aromatic acids were subjected to irradiation (7.3×10^{19} ev/g/h) in the solid state. Only the *p*-toluenesulfonic esters were altered; the substituted benzoic esters, regardless of the substitution on the benzene ring, were stable. Radiation change in the two *p*-toluenesulfonic esters occurred at the glycosidic bond, as shown by the i.r. spectra of the products. The stability of the other substituted D-glucosides was so high that, even after dosages as high as 5.2×10^{21} ev/g, no reducing power was measurable. The presence of either electropositive or electronegative substituents on the aromatic ring did not significantly affect the radiation change of the glycosidic bond, and it was suggested that protection arises mainly from intramolecular transfer of energy to the aromatic group, followed by dissipation of the energy as heat or light.

INTRODUCTION

Cleavage of the glycosidic bond is the predominant reaction when methyl α-D-glucopyranoside[1,2] and di-, tri-, and poly-saccharides[3] are exposed to high-energy radiation, both in the solid state and in aqueous solution. Phillips, Blouin, and Arthur[4] showed that a considerable amount of protection of the glycosidic bond can be achieved, over distances greater than one D-glucose residue, when aromatic esters of D-glucosides are subjected to γ-radiation. Protection appeared to be due to preferential transfer of energy to the aromatic group, with dissipation of the energy as heat or light.

The effects of the substitution of electropositive and electronegative groups (on the aromatic rings) on the stability of some of the properties of aromatic esters of the methyl D-glucopyranosides to high-energy radiation are now reported. Also, the synthesis and characterization of eight of these esters are given.

RESULTS

The glycosidic group in all of the compounds was found to be highly resistant

*Resident Postdoctoral Research Associate.

to radiation cleavage. No measurable amount of reducing power, as determined by the method of Somogyi[5], was found in any of the D-glucosides irradiated, even at dosages as high as 5.2×10^{21} ev/g. Moreover, after debenzoylation of the irradiated D-glucosides by alkaline hydrolysis[6], no reducing power was detectable. For methyl 2,3,4,6-tetra-*O*-*p*-tolylsulfonyl-β-D-glucopyranoside (**1**) and methyl 4-chloro-2,3,6-tri-*O*-*p*-tolylsulfonyl-β-D-glucopyranoside (**2**) under mild conditions of alkaline hydrolysis, desulfonylation was not accomplished, and complete dissolution was not possible. With methanolic sodium hydroxide or with sodium methoxide in methanol[7] under reflux, ring opening occurred, as indicated from the analysis of the unirradiated control.

In the i.r. spectra of irradiated **1** and **2**, a new band appeared at 1725 cm^{-1}, indicating the formation of carbonyl groups. For all of the other compounds, the i.r. and n.m.r. spectra were the same before and after irradiation. With the exception of **1** and **2**, the irradiated D-glucosides showed no component other than the starting material when examined by t.l.c.; irradiated **1** and **2**, showed an extra component (t.l.c.). Radiation-induced cleavage of the sulfonic ester linkage was not observed.

An estimation of the radiation decomposition of the glycosidic bond was made from the changes in optical rotation of the various compounds on irradiation. A sample of each irradiated D-glucoside was debenzoylated[6], and the optical rotation was measured; the value was compared with that for the debenzoylated control. The radiation decomposition for the D-glucosides at a dosage of 5.2×10^{21} ev/g was estimated to be as follows: methyl 2,3,4,6-tetra-*O*-nicotinoyl-α-D-glucopyranoside (**3**), 5.5%; methyl 2,3,4,6-tetra-*O*-(phenylcarbamoyl)-α-D-glucopyranoside (**4**), 1.0%; methyl 2,3,4,6-tetra-*O*-(*p*-methoxybenzoyl)-α-D-glucopyranoside (**5**), 2.2%; methyl 2,3,4,6-tetra-*O*-(*p*-nitrobenzoyl)-α-D-glucopyranoside (**6**), 2.2%; methyl 2,3,4,6-tetra-*O*-(*p*-ethoxycarbonylbenzoyl)-α-D-glucopyranoside (**7**), 5.5%; and methyl 2,3,4,6-tetra-*O*-(*o*-chlorobenzoyl)-α-D-glucopyranoside (**8**), 1.0%. The irradiated **1** had $[\alpha]_D^{25}$ −2.47° (*c* 0.8, acetone) as compared with $[\alpha]_D^{25}$ −9.7° (*c* 0.8, acetone) for the unirradiated sample. If the other D-glucosides irradiated were not debenzoylated prior to the determination, the changes in the optical rotation were not significant.

For all of the D-glucosides, irradiation induced long-lived free-radicals which were detected by the single line in their electron-spin resonance (e.s.r.) spectra; this indicated that the free radicals were, most probably, formed by dehydrogenation at the C-1–H bond. When the irradiated D-glucosides were stored under vacuum, the free radicals were still detectable several weeks after irradiation. When they were stored in air, the concentration of the radicals decreased; however, the shape of the e.s.r. spectrum did not change. The formation of stable free-radicals in carbohydrates on irradiation has been reported by other workers[8–10]. The color of the D-glucosides did not change on irradiation, except for **3**, which turned green; the green color increased in intensity with the dosage, and disappeared completely on storage in the air.

The fluorescence spectra of the D-glucosides changed very little on irradiation, as shown in Table I. In most cases, a new peak appeared in the longer-wavelength region

of the spectra. The intensity of the new peak increased with increase in radiation dosage.

TABLE I

FLUORESCENCE SPECTRA OF ESTERS OF METHYL D-GLUCOPYRANOSIDES[a]

Ester		*Before irradiation*		*After irradiation*	
		Activation peak, nm	*Fluorescent peak, nm*	*Activation peak, nm*	*Fluorescent peak, nm*
2,3,4,6-Tetra-*O*-(phenylcarbamoyl)-α-D-	(4)	277 (w)	302.5 (w)	341	405
2,3,4,6-Tetra-*O*-*p*-tolylsulfonyl-β-D-	(1)	306.6	370	309, 325	370, 450
4-Chloro-2,3,6-tri-*O*-*p*-tolylsulfonyl-β-D-	(2)	266.3	288.5	322	446
2,3,4,6-Tetra-*O*-(*p*-methoxybenzoyl)-α-D-	(5)	287	363.6	290	344
2,3,4,6-Tetra-*O*-(*p*-nitrobenzoyl)-α-D-	(6)	390 (vw)	442.6 (vw)	—	—
2,3,4,6-Tetra-*O*-(*p*-ethoxycarbonyl-benzoyl)-α-D-	(7)	300	375	310, 335	370, 400, 450
2,3,4,6-Tetra-*O*-(*o*-chlorobenzoyl)-α-D-	(8)	268 (vw)		264(vw)	
		297.7	331, 376	300, 365	333, 415
2,3,4,6-Tetra-*O*-nicotinoyl-α-D-	(3)	310	340	310 (vw)	336 (vw)
				339 (vw)	398 (vs)

[a]All of the spectra were measured in chloroform. [b]The spectra were determined 2–3 weeks after irradiation; key: s, strong; v, very; w, weak.

DISCUSSION

When carbohydrates in the solid state are irradiated by ionizing radiation, the mechanism of the loss of energy by the incident radiation to the carbohydrate molecule is probably an initial, random, nonlocalized acceptance of the energy, followed by dissipation of the energy as high-energy electrons within the molecule. The localization of the energy of these electrons is influenced by the presence of aromatic groups. This localization of energy could be effected through intramolecular transfer of energy to the aromatic group or selective absorption of energy by the aromatic group, or both. An aromatic system is able to accept the energy, to form well-defined, excited states; the energy could then be dissipated as heat or light without causing bond cleavage in the carbohydrate molecule. One simple way to deactivate the excited state of the aromatic group is by fluorescence. All of the substituted D-glucosides reported here exhibited fluorescence when excited by high-energy radiation. The intensity of the new fluorescence peak, which appeared after irradiation of the D-glucosides, increased with increase in radiation dosage.

For the *p*-toluenesulfonylated D-glucosides, **1** and **2**, the new band at 1725 cm^{-1} in the i.r. spectra was assigned to the carbonyl group, an intermediate formed during radiation damage of the glycosidic bond. The formation of this reducing group could be explained by slight delocalization of the aromatic, π-electron system by the *d*-orbital participation of the sulfur atom. This energy was

apparently insufficient to break the carbon–oxygen bond so as to cleave the *p*-toluenesulfonic group from the D-glucose residue.

Although intramolecular transfer of energy is the most probable path, intermolecular transfer of energy to the aromatic group is also a possibility. It has been shown that intermolecular transfer of energy occurs during the irradiation of carbohydrates[11].

EXPERIMENTAL

Melting points were determined in a Fisher–Johns apparatus, and were not corrected. Infrared (i.r.) spectra were recorded with a Perkin–Elmer Infracord spectrophotometer. Nuclear magnetic resonance (n.m.r.) spectra were determined for solutions in deuteriochloroform at room temperature, unless otherwise stated, with tetramethylsilane as the internal standard, by using a Varian A-60 spectrometer equipped with a V-6040 variable-temperature probe. The purity of the compounds was examined by thin-layer chromatography (t.l.c.) on Silica Gel G, with 1:1:3 (v/v) acetic acid–water–ethyl acetate for unsubstituted D-glucosides and 1:1 (v/v) ethyl acetate–petroleum ether (b.p. 30–40°) for the substituted D-glucosides. The spray reagent used was 5% sulfuric acid in ethanol (with subsequent charring for 10 min at 110°).

Methyl 2,3,4,6-tetra-O-(*phenylcarbamoyl*)-α-D-*glucopyranoside* (**4**). — This compound was prepared by a method described in the literature[12]. After recrystallization five to six times from alcohol–acetone, the product had m.p. 248–250° (dec)., $[\alpha]_D^{23}$ +72.9° (*c* 2.0, acetone); lit.[12] m.p. 227° (dec.), $[\alpha]_D^{23}$ +73.0° (*c* 3, acetone). ν_{max}^{KBr} 3390 (NH), a doublet at 1770 and 1730 (CO), 1618, 1466 (aryl C=C), 1548 cm^{-1} (Amide II); n.m.r. data (Me_2SO-d_6 at 52°): τ 0.24–0.9 (4-proton multiplet, NH); τ 2.49–3.57 (20-proton multiplet, aromatic); τ 4.32–5.49 (4-proton multiplet, pyranoside ring protons); τ 5.60–6.40 (3-proton multiplet, other ring proton and C-6-methylene protons); τ 6.75 (3-proton singlet, OMe).

Anal. Calc. for $C_{35}H_{34}N_4O_{10}$: C, 62.68; H, 5.07. Found: C, 63.25; H, 5.38.

Methyl 2,3,4,6-tetra-O-p-*tolylsulfonyl*-β-D-*glucopyranoside* (**1**). — This compound was prepared by the method described by Hess and Stenzel[13]; it had m.p. 183–4°, $[\alpha]_D^{23}$ −9.7° (*c* 0.7, acetone); lit.[13] m.p. 183–4°, $[\alpha]_D^{19}$ −9.8° (acetone). ν_{max}^{KBr} 1613, 1515, 1471 (aryl C=C), 1404, 1385 (-O-SO_2-), 1208, 1193 cm^{-1} (-O-SO_2-); n.m.r. data (Me_2SO-d_6 at 64°): τ 1.98–2.79 (16-proton multiplet, aromatic); τ 6.8 (3-proton singlet, OMe); τ 7.53 (singlet, aromatic methyl). Other protons could not be identified, because a poor spectrum was obtained owing to the insolubility of the compound.

Methyl 4-chloro-2,3,6-tri-O-p-*tolylsulfonyl*-β-D-*glucopyranoside* (**2**). — This compound was prepared by the method described by Hess and Stenzel[13]; it had m.p. 186–7°, $[\alpha]_D^{21}$ −18.7° (*c* 0.84, acetone); lit.[13] m.p.186–7°, $[\alpha]_D^{20}$ −18.9° (*c* 0.94, acetone). ν_{max}^{KBr} 1616, 1515, 1471 (aryl C=C), 1404, 1389 (-O-SO_2-), 1208, 1190 cm^{-1} (-O-SO_2-); n.m.r. data (Me_2SO-d_6 at 50°): τ 1.92–2.71 (12-proton multiplet, aromatic); τ 6.71 (3-proton singlet, OMe); τ 7.49 (singlet, aromatic methyl). Other protons could not

be identified, because a poor spectrum was obtained owing to the insolubility of the compound.

Methyl 2,3,4,6-tetra-O-(p-*methoxybenzoyl*)-α-D-*glucopyranoside* (5). — A solution of methyl α-D-glucopyranoside (4.8 g, 0.025 mole) in chloroform (20 ml) was cooled in ice, and a precooled solution (0°) of anisoyl chloride (25.5 g, 0.15 mole) and anhydrous pyridine (24 g, 0.3 mole) in chloroform (20 ml) was added, with stirring, at such a rate that the temperature did not rise above 0°. After the D-glucoside had dissolved, the mixture was kept in the ice-bath for 24 h, and then poured over ice-water, and the mixture was extracted thoroughly with chloroform. The extract was successively washed with ice-cold, dilute sulfuric acid, aqueous sodium carbonate, and water, dried (anhydrous sodium sulfate), and evaporated to a thick syrup which was dissolved in ether–chloroform. On cooling the solution, compound 5 crystallized (yield 80–85%). After two recrystallizations from the same solvent mixture, it had m.p. 144°; $[\alpha]_D^{25}$ +79.2° (*c* 2.0, acetone); ν_{max}^{KBr} 2857 (OMe), 1754 (CO), 1629, 1538 (aryl C=C), 1274 (O-CO-R) cm^{-1}; n.m.r. data: two sets of multiplets of a total of 16 protons (due to aromatic ring) centered at τ 2.01 (8 protons) and τ 3.11 (8 protons); τ 3.56–4.84 (4-proton multiplet, pyranoside ring protons); τ 5.17–5.75 (3-proton multiplet due to 1 ring-proton and 2 C-6-methylene protons); 4 singlets centered at τ 6.18 (12 protons, aromatic OMe); τ 6.48 (3-proton singlet, OMe).

Anal. Calc. for $C_{39}H_{38}O_{14}$: C, 64.10; H, 5.20. Found: C, 63.74; H, 5.10.

Methyl 2,3,4,6-tetra-O-(p-*nitrobenzoyl*)-α-D-*glucopyranoside* (6). — A solution of *p*-nitrobenzoyl chloride (12 g, 0.065 mole) in anhydrous pyridine (15 g, 0.19 mole) was added, with stirring, to a solution of methyl α-D-glucopyranoside (2 g, 0.010 mole) in anhydrous chloroform at 0°. The mixture was kept in an ice-bath overnight, and then processed as for 5. The solid product was recrystallized from alcohol–chloroform; (yield 80%); m.p. 181–2°; $[\alpha]_D^{25}$ +92.2° (*c* 1.0, acetone); ν_{max}^{KBr} 1761 (CO), 1626, 1550 (aryl C=C), 1366 (NO_2), 1290 (O-CO-R) cm^{-1}; n.m.r. data: τ 1.47–2.31 (16-proton multiplet, aromatic); τ 3.52–4.8 (4-proton multiplet, pyranoside ring protons); τ 5.22–5.6 (3-proton multiplet, other ring proton and C-6-methylene protons); τ 6.44 (3-proton singlet, OMe).

Anal. Calc. for $C_{35}H_{26}N_4O_{18}$: C, 53.16; H, 3.29. Found: C, 52.90; H, 3.41.

Methyl 2,3,4,6-tetra-O-(p-*ethoxycarbonylbenzoyl*)-α-D-*glucopyranoside* (7). — A solution of 10 ml of *p*-ethoxycarbonylbenzoyl chloride (prepared from the half-acid ester of terephthalic acid by the thionyl chloride method) was added, with stirring, to a solution of methyl α-D-glucopyranoside (1.5 g, 0.077 mole) in anhydrous pyridine (12 ml) at 0°, and the mixture was kept overnight at room temperature. The mixture, which had become dark red, was processed in the usual way, affording a dark-brown gum which did not crystallize, and was therefore purified by chromatography on neutral alumina. This treatment gave a pale-yellow oil which solidified on trituration with ether–petroleum ether (b.p. 30–40°). Several recrystallizations from alcohol gave colorless needles (yield 50–60%); m.p. 125°; $[\alpha]_D^{23}$ +69.3 °(*c* 2.0, acetone); ν_{max}^{KBr} 1761 (CO), 1592, 1520, 1471 (w, aryl C=C), 1290 (broad, -CO_2R-) cm^{-1}; n.m.r. data: τ 1.93, 1.98, 2.03, 2.09 (multiplets, 16 protons, aromatic); τ 3.67–4.88 (4-proton multiplet,

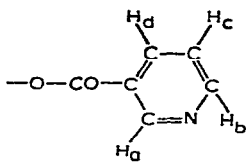
pyranoside ring protons); τ 5.28–5.88 (11-proton multiplet, 1 ring proton, 2 C-6-methylene protons and $8CO_2C\underline{H_2}CH_3$ protons); τ 6.46 (3-proton singlet, -OMe); τ 8.33–8.83 (12-proton multiplet, $-OCOCH_2C\underline{H_3}$).

Anal. Calc. for $C_{47}H_{46}O_{18}$: C, 62.80; H, 5.12. Found: C, 62.94; H, 5.17.

Methyl 2,3,4,6-tetra-O-(*o-chlorobenzoyl*)-α-D-*glucopyranoside* (**8**). — This compound was prepared from methyl α-D-glucopyranoside and *o*-chlorobenzoyl chloride by the method used for **7**, and the product was purified by chromatography on alumina. The resulting solid was recrystallized from alcohol–chloroform, giving a colorless, granular solid (yield 70%); m.p. 118–9°; $[\alpha]_D^{23}$ +76.6° (*c* 2.1, acetone); ν_{max}^{KBr} 1770 (CO), 1608, 1488, 1449 (aryl C=C), 1266 (CO_2R) cm^{-1}; n.m.r. data: τ 1.93–3.1 (16-proton multiplet, aromatic); τ 3.5–4.87 (4-proton multiplet, pyranoside ring protons); τ 5.16–5.8 (3-proton multiplet, other ring protons, and C-6-methylene protons); τ 6.55 (3-proton singlet, OMe).

Anal. Calc. for $C_{35}H_{26}Cl_4O_{10}$: C, 56.15; H, 3.47. Found: C, 56.45; H, 3.46.

Methyl 2,3,4,6-tetra-O-*nicotinoyl*-α-D-*glucopyranoside* (**3**). — Nicotinoyl chloride (9.0 g, 0.064 mole, prepared by the method of Wingfield *et al.*[14]) was added, with stirring, to a solution of methyl α-D-glucopyranoside (2.0 g, 0.010 mole) in a mixture of pyridine (10 ml) and chloroform (20 ml) at 0°. The mixture was kept overnight at room temperature, and then poured over ice, and the mixture was extracted with chloroform. The extract was extracted several times with cold, dilute sulfuric acid, and the aqueous extracts were combined and neutralized with dilute sodium hydroxide solution. An oil separated, and was collected by extraction with chloroform; the extract was washed with water, dried, and evaporated to dryness, giving a residue which was crystallized from alcohol–ether; fine needles (yield 60%); m.p. 142° (lit.[15] m.p. 137°); $[\alpha]_D^{23}$ +96.1° (*c* 2.0, acetone); ν_{max}^{KBr} 1754 (CO), 1613, 1497, 1441 (C=C and C=N, pyridine ring), 1307 ($-CO_2R$) cm^{-1}; n.m.r. data: four sets of multiplets of a total of 16 protons (due to the pyridine ring) centered at τ 0.84 (4 protons, H_a); τ 1.265 (4 protons, H_b); τ 1.74 (4 protons, H_d); τ 2.63 (4 protons, H_c); τ 3.47–4.84 (4-proton multiplet, pyranoside ring protons); τ 5.1–5.74 (3-proton multiplet, other ring protons, and C-6-methylene protons); τ 6.44 (3-proton singlet, OMe).



Anal. Calc. for $C_{31}H_{26}N_4O_{10}$: C, 60.59; H, 4.23. Found: C, 60.93; H, 4.53.

Irradiation. — The esters of methyl α-D-glucopyranoside in the solid state were irradiated in air at room temperature in the SRRL ^{60}Co radiation source[16] to the desired dosage. The dose rate, determined by ferrous–ferric dosimetry[17], was 7.3×10^{19} ev/g/h. The irradiated samples were analyzed immediately after irradiation.

ACKNOWLEDGMENTS

The authors thank Mr. G. J. Boudreaux for recording the n.m.r. spectra, and Mrs. N. M. Morris for recording the i.r. spectra.

REFERENCES

1 G. O. PHILLIPS AND G. J. MOODY, *J. Chem. Soc.*, (1960) 762.
2 M. L. WOLFROM, W. W. BINKLEY, AND L. J. MCCABE, *J. Am. Chem. Soc.*, 81 (1959) 1442.
3 G. O. PHILLIPS, *Advan. Carbohydrate Chem.*, 16 (1961) 13.
4 G. O. PHILLIPS, F. A. BLOUIN, AND J. C. ARTHUR, Jr., *Radiation Res.*, 23 (1964) 527.
5 M. SOMOGYI, *J. Biol. Chem.*, 195 (1952) 19.
6 L. B. GENUNG AND R. C. MALLAT, *Ind. Eng. Chem., Anal. Ed.*, 13 (1941) 369.
7 R. D. BROOKS AND N. S. THOMPSON, *Tappi*, 49 (1966) 362.
8 D. WILLIAMS, B. SCHMIDT, M. L. WOLFROM, A. MICHELAKIS, AND L. J. MCCABE, *Proc. Natl. Acad. Sci. U. S.*, 45 (1959) 1744.
9 D. WILLIAMS, J. E. GEUSIC, M. L. WOLFROM, AND L. J. MCCABE, *Proc. Natl. Acad. Sci. U. S.*, 44 (1958) 1128.
10 M. A. COLLINS, *Nature*, 193 (1962) 1061.
11 G. O. PHILLIPS, *Discussions Faraday Soc.*, 36 (1963) 281.
12 W. M. HEARON, *Methods Carbohydrate Chem.*, 2 (1963) 240.
13 K. HESS AND H. STENZEL, *Ber.*, 68 (1935) 981.
14 H. N. WINGFIELD, Jr., W. R. HARLAN, AND H. R. HAMMER, *J. Am. Chem. Soc.*, 75 (1953) 4364.
15 F. M. STRONG, L. LUTWAK, AND M. A. FAROOQUEE, *Arch. Biochem.*, 18 (1948) 297.
16 J. C. ARTHUR, Jr., F. A. BLOUIN, AND R. J. DEMINT, *U. S. Dept. Agr. ARS 72-21*, (1960) 7 pp.
17 R. H. SCHULER AND A. O. ALLEN, *J. Chem. Phys.*, 24 (1956) 56.